

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference <b>G62276PC</b>	<b>FOR FURTHER ACTION</b>	See item 4 below
International application No. <b>PCT/EP2004/008739</b>	International filing date ( <i>day/month/year</i> ) <b>04 August 2004 (04.08.2004)</b>	Priority date ( <i>day/month/year</i> ) <b>04 August 2003 (04.08.2003)</b>
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant <b>MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.</b>		

1.	This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).																								
2.	This REPORT consists of a total of 7 sheets, including this cover sheet.  In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.																								
3.	<p>This report contains indications relating to the following items:</p> <table style="width: 100%;"> <tr> <td style="width: 10%; text-align: center;"><input checked="" type="checkbox"/></td> <td style="width: 30%;">Box No. I</td> <td style="width: 60%;">Basis of the report</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td>Box No. II</td> <td>Priority</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td>Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td>Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td style="text-align: center;"><input checked="" type="checkbox"/></td> <td>Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td>Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td>Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td>Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table>	<input checked="" type="checkbox"/>	Box No. I	Basis of the report	<input type="checkbox"/>	Box No. II	Priority	<input type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	<input type="checkbox"/>	Box No. IV	Lack of unity of invention	<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	<input type="checkbox"/>	Box No. VI	Certain documents cited	<input type="checkbox"/>	Box No. VII	Certain defects in the international application	<input type="checkbox"/>	Box No. VIII	Certain observations on the international application
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4.	The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).																								

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No. +41 22 740 14 35	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="padding: 2px;">Date of issuance of this report <b>06 February 2006 (06.02.2006)</b></td> </tr> <tr> <td style="padding: 2px;">Authorized officer  <b>Agnes Wittmann-Regis</b></td> </tr> <tr> <td style="padding: 2px;">Telephone No. +41 22 338 89 70</td> </tr> </table>	Date of issuance of this report <b>06 February 2006 (06.02.2006)</b>	Authorized officer  <b>Agnes Wittmann-Regis</b>	Telephone No. +41 22 338 89 70
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Authorized officer  <b>Agnes Wittmann-Regis</b>				
Telephone No. +41 22 338 89 70				

# PATENT COOPERATION TREATY

From the  
INTERNATIONAL SEARCHING AUTHORITY

REC'D 17 DEC 2004

PCT  
WIPO PCT

To:

see form PCT/ISA/220

17/2

## WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing  
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference  
see form PCT/ISA/220

**FOR FURTHER ACTION**  
See paragraph 2 below

International application No.  
PCT/EP2004/008739

International filing date (day/month/year)  
04.08.2004

Priority date (day/month/year)  
04.08.2003

International Patent Classification (IPC) or both national classification and IPC  
C07K14/47; C07K14/435

Applicant  
MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER ...

### 1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

### 2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

### 3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



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**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
PCT/EP2004/008739

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**Box No. I Basis of the opinion**

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1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
  - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
  - a. type of material:
    - ☒ a sequence listing
    - ☐ table(s) related to the sequence listing
  - b. format of material:
    - ☒ in written format
    - ☒ in computer readable form
  - c. time of filing/furnishing:
    - ☒ contained in the international application as filed.
    - ☒ filed together with the international application in computer readable form.
    - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
PCT/EP2004/008739

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**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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**1. Statement**

Novelty (N)	Yes: Claims	3,12,16,19,20,22,26
	No: Claims	1,2,4-11, 3-15,17,18,21,23-25
Inventive step (IS)	Yes: Claims	
	No: Claims	3,12,16,19,20,22,26
Industrial applicability (IA)	Yes: Claims	1-26
	No: Claims	

**2. Citations and explanations**

**see separate sheet**

The following documents (D) are mentioned for the first time in this opinion/report; the numbering will be adhered to in the rest of the procedure:

D1: EP-A-1 238 982 (RIKEN) 11 September 2002 (2002-09-11)

D2: MIYAWAKI A ET AL: "Dynamic and quantitative Ca<sup>2+</sup> measurements using improved cameleons." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. 2 MAR 1999, vol. 96, no. 5, 2 March 1999 (1999-03-02), pages 2135-2140, XP002268872 ISSN: 0027-8424

**V) Novelty, inventive step and industrial applicability**

**Novelty**

- 1) The present application does not satisfy the criterion set forth in **Article 33 (2) PCT** because the subject-matter of **claims 1, 2, 4-11, 13-15, 17, 18, 21 and 23-25** is not new in respect of prior art as defined in the regulations (**Rule 64.1 - 64.3 PCT**).
- 2) D1 enablingly discloses fluorescent Ca<sup>2+</sup>-binding polypeptides comprising FRET donor-acceptor-pairs derived from a variety of fluorescent proteins (including CFP, GFP and YFP), a glycine-rich linker and troponin (see particularly page 3, lines 30-50).

The Applicant is requested to note that the range of troponin structures covered by the claims ("at least 80% to a 30 amino acid long polypeptide sequence...") is very large compared to the range of troponins already known to exist and encompassed by the disclosure of D1.

A selected sub-range is considered novel only if each of the following criteria is satisfied:

- i) The selected sub-range is narrow w.r.t. the range disclosed in the prior art;
- ii) the selected sub-range is sufficiently far removed from the end-points of the range disclosed in the prior art, as well as from any specific examples; and

iii) the selected range is not an arbitrary specimen of the prior art (a mere embodiment), but another invention providing a new technical teaching (a purposive selection).

Consequently, the broad sub-range of troponins referred to in the claims is not sufficient to endow the claims with novelty.

- 3) D1 also discloses the use of a nuclear localization signal (see page 11, lines 8-11) with said fluorescent Ca<sup>2+</sup>-binding polypeptides, as well as vectors, transformants and methods associated therewith (see page 7, line 52 - page 10, line 40). The Applicant is requested to note that placing a transformant in a liquid culture (see page 10, line) is sufficient to induce a change in the local Ca<sup>2+</sup> concentration (c.f. **claim 17**).

**Inventive step**

- 4) The present application does not satisfy the criterion set forth in **Article 33 (3) PCT** because the subject-matter of **claims 3, 12, 16, 19, 20, 22 and 26** does not involve an inventive step (**Rule 65.1 and 65.2 PCT**).
- 5) **Claims 16, 19, 20, 22 and 26** do not contain any additional features compared to the claims to which they refer that could render them inventive over D1. The various uses etc. claimed (e.g. to make transgenic animals or diagnostic kits, or to measure Ca<sup>2+</sup> concentrations in cell organelles) would be immediately obvious to the skilled person without further thought.
- 6) With respect to **claims 3 and 12**, D2 is considered to represent the closest prior art. It discloses fluorescent Ca<sup>2+</sup>-binding ("cameleon") polypeptides comprising (in order) CFP, calmodulin, a glycine-rich linker and YFP (see figure 1).

The difference between the cameleons of D2 and the subject-matter of **claim 3** of the present application is that in the latter, calmodulin is exchanged for a protein with similarity to human or chicken skeletal muscle troponin C.

Starting from D2, the objective technical problem may be seen as how to provide an alternative fluorescent Ca<sup>2+</sup>-binding protein.

D1 provides a solution to this problem by suggesting the use of troponin as an alternative to calmodulin for the calcium-binding portion (see page 3, line 49).

Consequently, the exchange of one Ca<sup>2+</sup>-binding moiety for another in the fluorescent Ca<sup>2+</sup>-binding polypeptides of D2 is seen as an arbitrary and, therefore, obvious one.

**General remarks**

- 7) The inventive step objection might be overcome by reasoned argument.

Furthermore, the novelty objection might be overcome by incorporating present **claim 3** into the main claim. This would also be consistent with the general teaching of application, particularly the examples and figures, which seem to be directed at ratiometric indicators rather than non-ratiometric ones.

It should also be noted that the dependency of **claim 19** on **claim 16** appears incorrect and that the "transgenic animal" in **claim 16** must be "non-human".